X-ray Structure Determination of a Camptothecin-resistant Point Mutant of Human Topoisomerase I in Complex with Camptothecin

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Abstract:

Human topoisomerase I (topo I) is the sole intracellular target of camptothecin (CPT) and other "topo I poisons," some of which are among the most promising anticancer drugs ever identified. However, the lack of high resolution structural information on drug binding is a major stumbling block in the design of new topo I poisons. We have developed protocols for the crystallization of human topo I in covalent complex with DNA, wherein crystallization depends on the presence of camptothecin. Surprisingly, CPT-dependent crystals can be obtained with the camptothecin-resistant point mutant N722S enzyme. In order to elucidate the binding mode of CPT, and the structural effects of CPT-resistant point mutants, Emerald BioStructures, has visited beam-lines X25 and X12B at the NSLS in order to collect high-resolution X-ray diffraction data sets (2.0 to 2.5 angstrom) on CPT-dependent N722S crystals that would otherwise only diffract to ~5 angstrom resolution using standard laboratory X-ray sources. During the NSLS 2000 fiscal year, we examined over 100 crystals at X25 and X12B and have collected a total of ~ 2000 oscillation images, one degree each, to arrive at ~10 data sets with 90-95% completeness to between 2.0 and 2.5 angstrom resolution. The data was processed on-site using Denzo HKL software. The crystal structure determination of the CPT-dependent N722S crystals via molecular replacement and multiple-isomorphous replacement methods have revealed the binding mode of CPT as well as the mechanism of drug resistance by the N722S mutation.

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